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AUG 07 2001

TECH CENTER 1600/2900

Application No. 09/782,672

Attorney's Docket No. 032705-002

libraries from organisms that cannot be cultivated and typically are isolated from extreme environments. The exotic microorganisms that inhabit scalding hot springs, freezing Arctic waters, sulfur-rich geothermal springs, highly saline waters, or extremely acid or alkaline habitats are called extremophiles, and their enzymes are dubbed extremozymes. Precisely because extremophiles thrive in such outrageous environments, they are tricky to grow in laboratory cultures, the conventional first step toward isolating products an organism secretes. One advantage of using prokaryotes, which include most extremophiles, is that their genes are linked together. So far, most of the extremozymes RBI has discovered have come from hyperthermophiles - microorganisms that thrive at temperatures that often exceed 100°C. This approach, pioneered by Recombinant Biocatalysis (Brennan, 1996), relies on the construction expression libraries by extracting DNA from samples and gene amplification by PCR™.

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Bypassing the culture hurdle, Recombinant BioCatalysis Inc. (RBI) in Sharon Hill, Pa., has gone straight to the target - shotgun cloning DNA from a mix of organisms to fast-forward discovery of the enzymes they produce. RBI's focus is on the discovery, development and commercialization of enzymes from microorganisms that live in biodiverse environments to provide biocatalysts for the pharmaceutical and chemical process industries, including glycosidases, lipases, aminotransferases, phosphatases, cellulases, esterases, catalysts for chiral resolutions and for peptide synthesis. The expression libraries are then screened by

brute force approaches that rely heavily on an accelerated robotic screening system that handles several different enzyme assays simultaneously and operates 24 hours a day.

Fast track to enzymes from extremophiles

Obtain biomass samples from extreme environments



Extract and purify DNA



Clone DNA segments to generate genomic gene expression libraries



Pool the expression products of several clones and screen for enzyme activity



Establish DNA sequence of gene encoding an enzyme



Subclone DNA sequence for large-scale expression of the enzyme



Optimize enzyme by random DNA mutagenesis.

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This technology promises to begin to tap the unexplored diversity of function in the natural world. However, it is intrinsically limited to catalytic activities that serve a biological